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Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 695 Huntington Ave., Boston, Mass.

Assistant Editor

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JAN 16 1952

NUTRITION REVIEWS

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TRENDS IN THE SCIENCE OF FOOD AND ITS RELATION TO LIFE AND HEALTH

It is clear that the health and economy of our own country and of the rest of the world will be under severe trials in the years ahead. There is little doubt among those who are watching world events that one of our greatest assets in dealing with the international tensions will continue to be our ability to assist, directly or indirectly, in the alleviation of hunger.

The related tasks, however, reach far beyond the basic requirement of preventing starvation among actual and potential allies. A primary goal at home and abroad is a much higher level of health that can be reached as a result of improved food practices. These advances also make possible higher standards of living in a very broad sense of economic stability and cultural progress. The rate of advance is dependent upon the twin requirements of research and education.

University faculties, students and the general public are increasingly aware of the divisive and restrictive elements in our national and international life. But there are also constructive elements at work. By supporting unselfishly the graduate and medical schools of our universities, the food industry has set a pattern of voluntary action that pays dividends—some are tangible, some are intangible, but, combined, they reach from our homes to the ends of the earth.

The fact that infectious and acute diseases such as pneumonia and diphtheria are coming under control rapidly has been cited with great emphasis at recent meetings of the leading medical societies and especially at this past year's meeting of the American Public Health Association. In contrast, much less progress has been made in finding the causes, other than just simply eating too much, that underlie the metabolic diseases such as hardening of the arteries, heart disease, diabetes, arthritis, premature aging and mental disorders. These are in part, at least, associated with faulty nutrition. They represent the greatest challenge in the field of public health in the years ahead.

The importance of the metabolic diseases is emphasized by the relative aging of our total population. Within the half century, the total population has been roughly doubled, but in the same period the number of our citizens above 60 years of age has quadrupled. It is not enough to survive into the years from 60 to 80, they should be years of health and enjoyment, and mental vigor.

A further trend in our population has largely escaped public recognition. It pertains to the greater physiologic demand for foods of high nutritive quality, compared to earlier decades—greater than in any known period in human history. Our population does not work hard, as it once did. Several millions of our citizens have dropped from a work output of about 3500 calories per day to about 2500 calories per day. For many of the nutrients, such as vitamins, minerals and proteins, the need per person is almost as great when doing light work as when working hard. Hence, unless our food supply is of higher nutritive quality than in earlier years, the modern television addicts and the fast-traveling automobile passengers will be living on a much lower plane of nutrition. In other words, less manual work lowers the caloric requirement and creates a need for diets of higher quality as represented by the "protective foods."

When Dr. Karl T. Compton, then President of the Massachusetts Institute of Technology, and the organizing committee of the trustees first talked with me ten years ago about plans for the Nutrition Founda-

tion, the clarity of purpose and the sincerity of the group were at once evident. By establishing the Foundation, the food industry sought to accomplish three closely related objectives:

(1) To assist the universities and medical schools in their endeavor to advance the frontiers of nutrition research;
(2) To assist in the graduate training of young scientists entering this general field of advanced study; and
(3) To give strength to unbiased educational measures that would be of greatest benefit to the public.

The Foundation's role in the ongoing scene, therefore, has been to find the individuals and the institutions where modest financial assistance would yield the greatest returns to the public. The objective has been to help the selected individuals in their respective attacks upon key, fundamental problems, rather than to divert their interest toward the solution of immediate problems on behalf of industry. The long-range sense of values shown by the trustees has been especially gratifying to research scientists and to educators. Without that insight on the part of trustees in regard to university graduate work, there would have been little prospect for the Foundation's success.

The Foundation's contribution to training young scientists should not be gauged solely by numbers. The numbers are significant, however, when it is recognized that these men and women have been selected and trained under the personal guidance of university faculty members with the highest standards of both research and good citizenship.

By September, 1951, the grants had assisted in the training of 169 post-doctorate research fellows, had enabled 196 graduate students to reach their doctorate degrees (Ph.D. or M.D.), had enabled 117 graduate students to reach their master's degree, and had furnished advanced training for 153 additional graduates beyond their B.S. degrees—a total of 635 young scientists who are likely to spend their lives in this area of work.

Again, the number of papers published in the scientific journals would mean little, except as they represent discoveries by the most highly qualified research men on our university faculties. The 828 scientific journal papers represent the kinds of exploration that these men select to explore as their own life investment. The universities are not being diverted from their best concepts of research and teaching. Instead, they are being aided by industry in shaping the foundation stones for broad scientific progress.

Without knowing what the human body actually needs, the scientists who guide agriculture, industry, government agencies, medical practice and education cannot safeguard and serve the public satisfactorily. They must have tests, also, by which to measure the things that are in food. An indication of the interest and progress in this field is given by the fact that more than one-half—roughly 500—of the papers with acknowledgments to the Foundation have included contributions to measuring the nutrients in foods or derived from them as they are used.

By far the most important and the most complex task facing nutrition scientists is to discover how foods function inside the body. That is the compass that will guide both agriculture and medicine in the future. Discovery of each new nutrient raises not one question, but many, because their functions are not simple, isolated phenomena—they form an exceedingly complex pattern in which all the nutrients are interrelated and must function in harmony or ill health results. In this broad sense, biologic and food scientists have only begun to make their contribution to human betterment.

No group of scientists can solve problems of the above magnitude in a few short years, but by systematically pushing the frontier forward wherever there is enough

light to blaze a new trail, advanced areas are opened, and practical applications follow at an amazing pace.

The degree of interest in research within the member companies may interest our readers: these companies have increased their own research staffs during the past ten years by approximately 300 per cent. A second bright spot on the horizon is the increasing number of firms that have a well-trained scientist in charge of research, with an administrative rank of Vice President or higher. Such recognition gives assurance that research receives serious consideration in building management policy. The task of safeguarding and improving the food supply involves more than laboratory tests based on nutrition. The housewife is quick to register her freedom to include consideration of flavor, color, time-saving and cost.

Before commenting upon the advances that have resulted from the Foundation's grants, it should be emphasized that many other organizations and individuals should be mentioned for their joint contributions, if space permitted. Nevertheless, it is a recognized fact that we have aided, in a substantial way, the investigators who have accomplished the following types of advance in the space of a few years:

Scientists have learned to measure and use intelligently two new vitamins, folic acid and vitamin B_{12}. These two normal constituents of protective foods are recognized by the medical profession the world over. Food and animal feed manufacturers include them among the most important items to be retained in or added to their products. Otherwise, anemia, nervous disorders, intestinal infections, poor growth, and other evidences of poor health result.

Extensive headway has been made in discovering why good nutrition is normally the *major factor* in furnishing protection against tooth decay. Good diets during gestation and early childhood, we have reason to believe, would prevent 80 per cent or more of the tooth decay that is so prevalent. The extent of this problem is shown by the estimate of dentists that their entire profession working full-time could take care of only about one-third of the children who need dental care.

The addition of fluorides to public water supplies represents a major advance in combatting tooth decay, but it does not solve the entire problem.

The first and almost the only record available of the human protein requirement in terms of each individual amino acid has been established and has already guided an extensive amount of medical and food research. This basic datum on protein requirements is especially timely in the light of new discoveries that show how quickly the body functions are distorted from their normal activity when good quality proteins are omitted from the diet. The disturbances are evident within one day, and severe within a few days.

As a result of work in eight universities, new pathways are known by which sugars are burned or converted to other body constituents. This work includes both the normal conditions in men and in animals and also many disturbed conditions as in diabetes.

The role of fats in human health has leaped into sudden prominence. In this area of research, some of the grantees have made very significant progress. Originally they were simply studying how fats and oils are built up or broken down in normal living cells, but almost at once these changes are entering the arena of life-and-death statistics and the use of food. Overweight individuals are notably unpopular with insurance companies —and justly so.

The fats that are deposited in the arteries, liver and kidneys with the onset of hardening of the arteries are especially interesting. The worst offender in this series of discoveries is the familiar pattern of too many calories and too little of other essentials. The basic research on these complex fats has done

more than help the medical profession in fighting one of the most destructive diseases. It has had an important role in stemming the wave of hysteria about the risk of eating a reasonable amount of such foods as milk, meat and eggs. Not realizing that the body's total supply of the deposited fatty material, cholesterol, is chiefly dependent upon the total calories and fat eaten from any source, it was natural that many persons were mistakenly alarmed at the risk of consuming the small amounts found in common foods such as meat, milk and eggs. As a matter of fact, there is much good evidence to show that normally an adequate intake of the animal protein foods is one of the most important means of avoiding fatty livers and a great number of related injuries to health.

One of the strongest trends in current nutrition research is the emphasis, based on clear-cut evidence, that both the essential and nonessential nutrients are in constant interplay, within the body, and hence their quantitative requirements are interdependent. One must view the quality of the diet as a whole and avoid the extremes and imbalances that are so often encountered by getting excited about only one or a few vitamins or minerals.

It is utterly foolish to emphasize too greatly the role of only one or a few nutrients such as a single mineral, sugar, protein or vitamin, or the nutrition of a single part of the body, such as the hair or skin. That is why nutrition scientists are so heartily in agreement with our trustees in believing that the only reasonable goal in nutrition is a lifetime concept of good food habits.

CHARLES GLEN KING,
Scientific Director
The Nutrition Foundation, Inc.

VALUE OF D-METHIONINE ON A LOW NITROGEN INTAKE

In establishing the quantitative requirements for the essential amino acids for adult rats it was assumed that the unnatural isomer of methionine (D-methionine) was utilized (*Nutrition Reviews* **8,** *248 (1950)*).

The precise role of D-methionine under conditions of low nitrogen intake was recently investigated by E. S. Nasset and J. T. Anderson (*J. Nutrition* **44,** *237 (1951)*). These workers measured the relative effectiveness of DL- and L-methionine in adult rats by the nitrogen balance method. The amino acid diets used were supplemented with the known B vitamins except vitamin B_{12}. These diets contained no cystine or cysteine. They provided 11.5 mg. of choline chloride per rat daily.

The animals used in the study each received 43.9 calories daily. Uniform intake was assured by feeding amino acid diets twice a day with a stomach tube.

Each experiment consisted of the following sequence of feedings: (1) maintenance diet containing 9.6 per cent of whole egg protein, two weeks; (2) a nitrogen-free diet, one week; (3) the amino acid diet containing approximately one-half the nitrogen requirement, one week; and (4) the amino acid diet containing approximately the maintenance requirement for nitrogen, one week. This five-week cycle of feeding was repeated for each amino acid mixture under study.

The amino acid mixture used was similar to whole egg protein, except for methionine, with respect to the natural isomers (L-forms) of the essential amino acids. The nonessential amino acids were supplied by the D-forms of some of the essential amino acids and by sufficient L-glutamic acid to make the mixture equal to whole-egg protein in total nitrogen.

The diet supplying one-half of the nitrogen requirement for maintenance contained either 22.7 mg. of DL-methionine nitrogen

per gram of total nitrogen or an equal amount of L-methionine. Hence the latter contained twice as much of the natural isomer as the former.

The diets supplying enough amino acids to meet the approximate maintenance requirement for nitrogen contained 45.4 mg. of either DL- or L-methionine nitrogen per gram of total nitrogen.

The nitrogen balance data were expressed in terms of body weight in kilograms to the three-fourths power, an estimate of the metabolic body size.

The results indicated that when the amino acid diets supplied one-half the nitrogen requirement, the natural isomer (L-methionine) permitted significantly greater nitrogen retention than did the racemic mixture (DL-methionine). The animals receiving the L-methionine excreted 159 mg. of nitrogen per day per kilogram body weight$^{0.75}$ while those receiving the DL-methionine excreted 194 mg. Both groups were ingesting 74 mg. of nitrogen daily per kilogram body weight$^{0.75}$.

When the diet contained approximately the maintenance requirement for nitrogen, the L- and DL- forms of methionine had equal activity. In both cases the daily nitrogen excreted exceeded the intake by 31 mg. daily per kilogram body weight$^{0.75}$.

As the authors point out, it is paradoxical that an animal in severe negative nitrogen balance should fail to utilize the D-form of methionine and yet apparently be able to utilize this form of the amino acid at or near nitrogen equilibrium.

In view of the report that liver enzymes are greatly reduced when rats are fed diets low in protein (L. L. Miller, *J. Biol. Chem.* **172,** *113 (1948)*), a possible explanation for these results might be a diminished synthesis or function of enzymes that are concerned with the utilization of D-forms of amino acids. If such were the case, it would be of interest to determine the relative effectiveness of the D-form of methionine in severely protein-depleted animals. In studies dealing with the maintenance requirement and the requirement for the rebuilding of protein in protein-depleted adult rats, it was noted that the DL-methionine requirement for protein rebuilding was considerably greater than that for maintenance (*Nutrition Reviews, loc. cit.*).

The results of Nasset and Anderson are of further interest in view of the recent report that D-methionine was only 76 to 84 per cent as effective as L-methionine for the support of growth in young rats (K. A. J. Wretlind, *Acta. physiol. Scandinav.* **20,** *1 (1950)*).

From the nitrogen balance index Nasset and Anderson calculated that the adult rat would require approximately 40 mg. of methionine daily per kilogram body weight$^{0.75}$ (110 mg. per kilogram body weight), which compares favorably with earlier reported values for maintenance (*Nutrition Reviews, loc. cit.*).

SODIUM RESTRICTION IN HYPERTENSION

In a recent review of the dietary treatment of hypertension, (*Nutrition Reviews* **9,** *73 (1951)*) it was observed that approximately one-third of 75 patients treated with the rice diet had a clear-cut reduction in blood pressure, and it was emphasized that the mechanism of the effectiveness of the diet required study. In this same review, the work of V. P. Dole *et al.* was discussed with particular reference to metabolic and balance studies of these patients. These authors noted a loss of weight during the initial period on the rice diet which then stabilized. Sodium and potassium equilibrium were found to occur after stabilization on the rice and fruit regime.

V. P. Dole and co-workers (*J. Clin. Invest.* **30,** *584 (1951)*) have continued their studies of the metabolic changes during the dietary treament of hypertension. Five patients with "benign, uncomplicated hypertension" and 3 normal individuals were investigated. The 3 normal individuals were used as standards for exchangeable sodium determinations in the patients. The studies were continued from October 1949 through April 1950.

The study for each patient was divided into six periods of from eighteen to forty-seven days duration each. A diet was provided furnishing less than 7 mEq. of sodium per day. During some of the periods the sodium intake was increased by the addition to the diet of salt tablets which furnished 180 mEq. per day. Water was restricted during some of the periods just to the point of thirst, while during other periods 4500 ml. of water were given in addition to that contained in the diet. The diet furnished between 1600 and 3400 calories per day which varied from patient to patient, and varied somewhat during each patient's study. The protein intake was maintained at approximately 40 g. per day throughout the study, except during a period and a half for each of the patients, when the protein intake was increased to about 60 g. per day. In addition to regular measurements of blood pressure and heart size, the quantity of "exchangeable sodium" was estimated and compared with the body weight. The exchangeable sodium was the total amount of body sodium in equilibrium with a known amount of Na^{24} which was administered for the test.

All but one of the 5 patients were maintained on 180 mEq. of sodium from October through January. During the second period (November and December), the water intake was reduced, a high fluid intake was given during January and February and then a low fluid intake during the last periods of study. The final period of study was a high sodium intake again in all but one of the patients.

In all instances in which the daily sodium was reduced from 180 to 7 mEq. there was a significant fall in blood pressure, usually occurring in the first period after sodium restriction, and in most instances rising again in the final period when sodium was added to the diet. There was a distinct and significant reduction in cardiac size in 2 of the patients following salt restriction and an increase in heart size when sodium was replaced in the diet for 3 of the patients. A distinct and significant reduction in the total body content of exchangeable sodium also occurred following salt restriction in all 4 of the patients in whom this measurement was pertinent. Likewise, a significant rise in sodium occurred when salt restriction was stopped and 180 mEq. of sodium were given again. These effects appeared to be independent of the caloric and protein contents of the diet and of the amount of water administered.

Weight loss occurred in all of the patients studied, and was more rapid at first, then began to level off somewhat, but did not reach a constant figure at any time for any of the patients, except perhaps during the high protein feedings. It was considered by the authors that the weight loss was most probably due to limitation of protein in the diet.

Upon changing from the high to the low sodium diet, weight loss was not proportional to sodium loss. Since the serum sodium concentration did not change significantly, an exchange of water into the cells is implied by the data. Thus, sodium depletion appeared to reduce the quantity of exchangeable sodium, presumably mostly in the extracellular fluid, to reduce the volume of extracellular fluid, but caused an increase in cellular hydration amounting to about 5 per cent. When salt administration was reinstituted, cellular hydration again decreased as sodium was retained in the extracellular fluid. The authors considered that the reduction in blood pressure which occurred during salt restriction was most closely related to the increase in cellular hydration, but pointed out that more precise observations would have to be provided before this could be accepted as fact.

INFLUENCE OF FLOUR ENRICHMENT ON RAT GROWTH

Investigators at Kansas State College have evaluated the effects of flour enrichment on rats that were fed diets comparable to those consumed by humans (B. D. Westerman and M. Stone, *J. Nutrition* **44,** *313 (1951)*). The experiments were carried through two or more generations to determine the effects of long-continued usage of diets relatively high in bread, either enriched or nonenriched.

In an earlier study (B. D. Westerman, D. R. Linn, F. Templeton, and R. I. Wells, *J. Nutrition* **38,** *421 (1949)*) it was found that the use of enriched white flour in diets comparable to human diets promoted better growth in rats than the use of unenriched white flour.

In the more recent study by Westerman and Stone two different diets were used. One was based on data of average food consumption of low-income groups in South Carolina and the other was patterned after food consumption data on sixth-grade school children gathered in certain communities in Kansas.

To facilitate the preparation of the diets, foods were grouped as follows: Apples served as fruits in the diet; dried beans and peas as legumes; carrots as the yellow vegetables; and spinach, broccoli, cabbage and string beans served as green vegetables. The cereal ingredient was either enriched or nonenriched bread, and comprised approximately 50 per cent of the calories in the diet.

The composition of 924 g. of the prepared diet (based on the South Carolina low-income human diet) was as follows: 91.4, 69.4, 343 and 1.06 g. of protein, fat, carbohydrate and calcium respectively; 10,093 I.U. of vitamin A and 80 mg. of vitamin C; 924 g. of this diet contained 2374 calories. The diet containing the bread ingredient in nonenriched form contained the following in milligrams per 924 g. of diet: iron, 22.4; thiamine, 1.12; riboflavin, 1.19; and niacin, 8.0. The diet containing the enriched bread contained these ingredients in the following amounts (milligrams per 924 g.): iron, 29.4; thiamine, 3.11; riboflavin, 1.95; and niacin, 19.7.

The growth tests were conducted on weanling rats over a period of ten weeks. In the first experiment the diet used was that based on the South Carolina human food consumption data. In the first generation the animals with enriched flour in the diet gained an average of 15 g. more than those with nonenriched flour in the diet. In the second generation a more pronounced effect from enrichment was evident. A total average gain of 225 g. was obtained on the enriched flour diet compared with 164 g. on the nonenriched flour diet.

After completion of the growth tests the rats were bred in order to obtain data on reproduction and lactation performance. The first mating of rats receiving the nonenriched flour diet weaned 72 per cent of their young whereas those receiving the enriched flour diet weaned 88 per cent of their young, the number of young at birth being equal.

The second mating produced somewhat more pronounced results. The animals on the enriched flour diet not only weaned a larger percentage of their young but also produced larger litters. Furthermore, the mothers receiving the nonenriched flour diet lost considerable weight during the lactation period and the fur of the young was rough and matted. The weaning weights of the young were uniformly between 50 and 60 g. and did not seem to be influenced by the dietary treatments.

Young from the first matings served as parent stock for the second generation studies. The reproduction and lactation performances in the second generation were essentially similar to those noted in the first generation. The animals receiving the enriched flour diet produced more young and weaned a larger percentage from first matings than did those receiving the nonenriched flour diet. The latter weaned only 63 per cent of their young from the second mating and

the mothers were noted to be in inferior physical condition after the lactation period.

Essentially similar results were obtained with the diet based on the food consumption habits of sixth-grade children in Kansas. This diet produced lower gains in weanling rats, apparently due to its somewhat lower protein content. The data indicated that this diet was probably inferior to the diet used earlier since reproductive failure resulted from the third mating of the first generation in the animals receiving the nonenriched flour.

The authors point out that the superior growth and the better reproduction and lactation performances of rats receiving the enriched flour diets may be directly attributable to the contribution of the B vitamins and the iron in the enriched flour.

Studies of this type seem desirable since too often human nutritional studies neglect to consider the possible long-term effects of a particular dietary. Long range effects of enrichment programs in human populations are extremely difficult to evaluate, as was pointed out in an earlier review (*Nutrition Reviews* **7,** *331 (1949)*).

From the data presented, and if analogy may be drawn for the human dietary, it is possible that the benefits of cereal enrichment may express themselves more extensively in future generations.

THYROIDECTOMY AND VITAMIN D IN PRODUCTION OF ARTERIOSCLEROSIS

The problem of the experimental production of atherosclerotic lesions in the experimental animal and the relationship of these to similar lesions in the human have been subjected to intensive but largely inconclusive study in many animals. From the standpoints of gross appearance and distribution of the lesions, satisfactory atherosclerosis has been produced in the dog by A. Steiner, F. E. Kendall, and M. Bevans (*Nutrition Reviews* **8,** *74 (1950)*); *Am. Heart J.* **38,** *34 (1949)*). These workers found that cholesterol and thiouracil produced arterial lesions in a period of twelve months or more. In their experience, thiouracil or cholesterol alone over even longer periods did not result in vascular lesions. Previous workers (N. Goormaghtigh and H. Handovsky, *Arch. Path.* **26,** *1144 (1938)*) had demonstrated chronic calcified areas, in the media of the aorta, in dogs and other animals receiving toxic doses of vitamin D. This appeared to be accentuated by thyroidectomy. Whether a specific arterial lesion is produced prior to the medial calcification has been debated by other investigators who have studied the process in the rat (J. B. Duguid, *J. Path. Bact.* **33,** *697 (1930)*; A. W. Ham, *Arch. Path.* **14,** *613 (1932)*). In an attempt to study the early lesions of atherosclerosis W. B. McAllister and L. L. Waters (*Yale J. Biol. Med.* **22,** *651 (1950)*) have used thyroidectomized dogs receiving viosterol.

In this study, 20 thyroidectomized dogs were fed 50,000 units of viosterol in oil per kilogram of body weight daily mixed with a diet of kidney meat. Eight normal dogs given viosterol and an unspecified number of thyroidectomized dogs not given viosterol served as controls. The experimental animals were examined at autopsy by groups, 8 dogs receiving the viosterol for four weeks or less, 6 for four to ten weeks and 6 for ten or more weeks. The authors state that no gross vascular lesions were found prior to fourteen days of viosterol administration and that between two and four weeks the vessels showed focal scattered areas of medial damage. These areas became widespread and confluent in the dogs treated for longer periods. During the first ten days of viosterol administration, microscopic examination revealed edematous areas of the media of the aorta, and after two weeks or more of treatment focal necrosis of the media with destruction of the elastic fibers appeared. This

was accompanied by inflammatory reactions about the necrotic areas. Subsequently intimal proliferation occurred and new capillaries were seen invading the outer areas of the media. Late in the process calcium salts were observed in medial areas such as these, and no inflammatory reaction could be observed.

Distribution studies of the vascular damage thus produced revealed that the root of the aorta, the thoracic aorta and the pulmonary artery were most heavily and most consistently involved. Next in frequency were damage of the left auricular endocardium and the abdominal aorta. Calcification of the latter and the finding of intimal proliferation of the coronary arteries were most striking in their increasing incidence as the duration of the experimental period was increased. In addition, the coronary arteries in dogs given viosterol for from ten to twenty weeks were often found to possess lipid-containing foam cells similar to those seen in human arteriosclerosis. These cells were frequently but not always associated with marked elevation of serum lipids.

Vascular lesions were found in the control dogs fed viosterol, but in fewer numbers with less acute and less extensive microscopic changes. Although the distribution was similar to that in the experimental animals, no gross lesions were seen until after six weeks of vitamin D and no foam cells were seen in animals killed at ten weeks. Atherosclerosis was not found in the thyroidectomized controls.

The blood chemistry changes usually found in vitamin D intoxication (elevated calcium, phosphorus, lipid, and nonprotein nitrogen in the serum) were noted in all animals receiving viosterol, but thyroidectomized animals showed more marked aberrations than did the normals. The authors state that cholesterol and fatty acid levels at least doubled their values during viosterol administration, although figures for all animals are not given. From the data given on 3 experimental animals and one thyroidectomized control it appears that most of the chemical changes tended to stabilize after two weeks or more, although values remained elevated. This was true of both types of dogs, and suggests the presence of compensatory or interfering mechanisms.

Although the findings of this study are of interest in regard to the production of vascular lesions similar to those of human arteriosclerosis, several points deserve comment. The association of hyperlipemia and vascular lesions in this study is suggested by the authors. However, other mechanisms cannot be excluded on the basis of the data presented. The presence of focal calcification in the basement membrane and epithelial cells of the renal tubules, of calcified casts in the collecting tubules, and focal vascular lesions of the kidney, as described in this report, all suggest the existence of renal disease. Involvement of this organ may have been as important as the alterations in lipid metabolism in producing the generalized atherosclerosis.

Another important consideration which is not commented upon by the authors is the effect of thyroidectomy and vitamin D intoxication upon food intake. Both of these factors tend to reduce the amount of food eaten by animals and accordingly, a decreasing intake of the diet and viosterol may have occurred. This would lead to an explanation for the stabilization of blood chemical changes observed in the animals as the studies were prolonged. It is also possible that the progress of the vascular damage was slowed by diminishing food and vitamin D intake. Under these circumstances a forced feeding regimen might have accelerated the changes.

This study presents a method for producing early atherosclerotic lesions in the dog which are similar to those of the human. The method has the advantage of doing so in the relatively short time of six to ten weeks in comparison to the twelve months required by the method of Steiner and Kendall (*loc. cit.*). The importance of the thyroid in pro-

tecting against such vascular disease is suggested by this study, but the mechanisms involved in the production of atherosclerosis appear to require more refined investigation, perhaps involving modifications of the viosterol and thyroidectomy technic.

NONESSENTIAL AMINO ACIDS AND NITROGEN UTILIZATION

The philosophy of the indispensable amino acids is in harmony with that of Liebig's law of the minimum, *i.e.*, the results of pertinent investigations to date show what must be the minimum qualitative make-up of the nitrogenous part of the experimental diet to support growth in the young or maintenance in the adult mammal. Thus there are listed ten amino acids essential for growth of the young rat, nine required for maintenance of the adult rat, but only eight which are necessary for the support of nitrogen balance in adult man. It is obvious that the individual subsisting on an ordinary mixed diet consumes compounds of nitrogen that are not included in the list of essential amino acids, and indeed, may have little if any structural chemical relationship to this group of dietary components. The question arises as to what may be the influence of such nonprotein nitrogenous substances upon the utilization of amino acid nitrogen. Or, inasmuch as the eight amino acids essential for nitrogen balance in man account for only 40 to 50 per cent of the composition of proteins of the highest biologic value, is it likely that the nonessential amino acids enhance the metabolic value of the mixture?

That the essential amino acids, though indispensable for growth and maintenance, will not support optimal growth when providing the sole source of nitrogen in the ration has been reported repeatedly (*see* G. B. Ramasarma, L. M. Henderson, and C. A. Elvehjem, *J. Nutrition* **38,** *177 (1949)*; W. C. Rose, L. C. Smith, M. Womack, and M. Shane, *J. Biol. Chem.* **181,** *307 (1949)*). Likewise, there is convincing evidence that several nitrogenous compounds not only are utilized themselves but apparently enhance the utilization of amino acid nitrogen. Thus it has been observed that the nonessential amino acids render a diet, whose nitrogen is derived from the essential amino acids, distinctly superior in promoting growth of young rats. Furthermore, either L-glutamic acid or diammonium citrate in isonitrogenous amounts with that of the nonessential amino acids will also effectively supplement the essential amino acid nitrogen for growth (Rose *et al.*, *loc. cit.*). In another study (H. A. Lardy and G. Feldott, *J. Biol. Chem.* **186,** *85 (1950)*) it was found that the retention of nitrogen was actually a little higher when the basic essential amino acids were supplemented with diammonium citrate than when the supplementary nitrogen was provided by the nonessential amino acids.

A recent study (D. V. Frost and H. R. Sandy, *J. Biol. Chem.* **189,** *249 (1951)*) was planned to determine whether or not the nonessential amino acids are necessary for optimal utilization of dietary nitrogen, and if so, what proportion of the total nitrogen of the diet should be present in forms other than essential amino acid nitrogen.

The rat repletion method was used (D. V. Frost and H. R. Sandy, *Proc. Soc. Exp. Biol. Med.* **68,** *51 (1948)*); the nonprotein depletion diet contained only 0.03 per cent nitrogen and supplied 4.2 calories per gram. In one test the amino acid mixture was patterned after the composition of casein; it consisted of the ten essential amino acids to which were added, in different trials, various combinations of L-glutamic acid, DL-aspartic acid or glycine and DL-alanine, fed in 5 per cent solution to supply 0.24 g. of nitrogen per rat per day. On the basis of gain in body weight during repletion and of acceptability of the amino acid mixture, it appears that

glutamic acid was the most effective adjuvant to the ten essential amino acids.

In another test with twice the minimum levels of nine essential amino acids as the nitrogen source it was found that the response was poor, but was improved equally by adding arginine, glycine or a mixture of alanine and glycine. It was concluded that the requirement for nonessential amino acid nitrogen was a real one but that it was relatively nonspecific. Further studies in which urea or ammonium citrate were compared with glutamic acid showed that ammonium citrate enhanced the utilization of the total amino acid nitrogen better than did urea and about the same as did glutamic acid.

In these latter experiments the proportion of nonspecific nitrogen to essential amino acid nitrogen was about 33 per cent; other ratios of substitution were made with nonessential amino acids and with ammonium acetate. The data suggest that to promote efficient utilization of the nitrogen of an amino acid mixture at least 20 per cent of the nitrogen should be in a form other than that of the essential amino acids. These observations raise questions with respect to the relative metabolic significance of the essential amino acids on the one hand, and the nonspecific nitrogenous adjuvants on the other, questions which require further investigation before they can be answered.

INFLUENCE OF RAW SOYBEANS ON VITAMIN A STORAGE IN CALVES

Several reports have appeared which indicate that the feeding of raw soybeans or soybean hay produces symptoms of vitamin A deficiency in dairy cattle (J. H. Hilton, S. M. Hauge, and J. W. Wilbur, *J. Dairy Sci.* **18,** *795 (1935)*; J. C. Shaw, L. A. Moore, and J. F. Sykes, *Ibid.* **30,** *567 (1947)*; R. L. Squibb, C. Y. Cannon, and R. S. Allen, *Ibid.* **31,** *421 (1948)*; and Shaw, *Ibid.* **33,** *486 (1950)*).

An experiment was reported recently in which quantitative data were obtained on blood plasma carotene and vitamin A, and liver vitamin A levels in calves fed soybeans in a ration of known carotene content (J. C. Shaw, L. A. Moore, and J. F. Sykes, *J. Dairy Sci.* **34,** *176 (1951)*).

The 4 calves used in the study, 2 Ayrshires and 2 Holsteins, were placed on a vitamin A deficient diet at 14 days of age. They were considered depleted when blood vitamin A had decreased to less than 5 micrograms per cent of plasma. Each calf was then given in addition to the basal diet 32 micrograms of carotene per pound of body weight per day in the form of alfalfa leaf meal, or slightly more than the minimum requirement, for the remainder of the experiment. After two weeks on carotene supplement, 2 calves (one Ayrshire and one Holstein) were continued on the basal diet while the remaining 2 calves were changed to the soybean test diet. The basal diet contained the following in pounds: barley, 240; oats, 180; wheat bran, 180; linseed meal, 60; and salt, 8. The soybean test diet was the same except that 200 pounds of raw soybean replaced the linseed meal and 140 pounds of the barley. Each diet was fortified with 75 g. of D-activated plant sterol. Each calf also received daily 1 pound of skim milk.

Both the plasma carotene and the vitamin A values were significantly lowered in the calves receiving the soybean test diet; the vitamin A values were lowered more than the carotene values.

Average carotene values (micrograms per cent of plasma) were 24.0 for the calves on the basal diet and 18.0 for calves on the soybean test diet. Corresponding vitamin A values were 9.3 and 6.0 for control and soybean fed calves respectively.

The vitamin A of the liver at the end of a one hundred and seventy-day experimental period was even more strikingly influenced by soybean feeding. The control calves had

4.07 and 9.22 micrograms of vitamin A per gram of liver, whereas the 2 calves on the soybean diet had liver vitamin A values of 0.72 and 2.75 micrograms per gram.

The authors point out that from a practical point of view it may be hazardous to use soybeans in the ration of cattle, particularly if such cattle received poor roughage of low carotene content, such as low quality timothy hay.

No explanation was offered concerning the possible mechanism of action of the raw soybeans in lowering the blood levels of vitamin A and carotene and in reducing liver storage. In this connection, it is of interest that soybeans are claimed to be a potent source of carotene oxidase (lipoxidase), an enzyme that is capable of destroying carotene by oxidation (R. Reiser and G. S. Fraps, *J. Assn. Official Agric. Chem.* **26,** *186 (1943)*).

Reproductive disturbances have been reported from the feeding of soybean hay to rabbits (K. A. Kendall, G. W. Salisbury, and N. L. Vandemark, *J. Nutrition* **42,** *487* (1950)). These workers considered a vitamin A shortage, brought about through enzymatic destruction of carotene in the soybean hay by lipoxidase, as an explanation for the reproductive disturbances. In reviewing the literature these investigators pointed out that a similar explanation might account for the reproductive disturbances that have been reported in sheep fed soybean hay.

LIPOTROPIC FACTORS AND PHOSPHOLIPID TURNOVER IN HEPATITIS AND ADHESIVE PERICARDITIS

The relationship between choline and methionine and the rate of phospholipid turnover as measured by the use of radioactive phosphorus has been the subject of extensive investigation by W. E. Cornatzer and D. Cayer and their colleagues. Initial studies in their clinic have revealed a significant increase in phospholipid turnover following the administration of choline or methionine to patients with hepatic cirrhosis. This effect is apparent before therapy in such patients, but is not demonstrable when treatment has been continued for several months (*Nutrition Reviews* **8,** *284 (1950)*). Subsequent to this work, which suggests that a deficiency of lipotropic factors exists in untreated cirrhosis, studies have been carried out in an attempt to determine whether a similar deficiency is also present in patients with hepatitis (D. Cayer and W. E. Cornatzer, *Gastroenterology* **18,** *79 (1951)*).

Phospholipid turnover was measured in 6 patients with hepatitis (4 infectious, 2 homologous serum in type) during the first week of their illness. All had been receiving good diets prior to the onset of symptoms. All received 10 g. of either choline chloride orally or methionine intravenously coincident with the administration of radioactive phosphorus. This procedure was repeated after several months of treatment and the two curves of phospholipid turnover were compared.

In 4 patients the specific activity of the phospholipid fraction increased with the initial dose of lipotropic factor in comparison with the posttreatment phospholipid turnover. However, when the results were calculated as relative specific activity (thus taking into account changes in inorganic- as well as phospholipid-phosphate specific activity) the differences became much smaller and were, except for one patient, not significant. The patient who did demonstrate increased phospholipid turnover is stated to have had severe anorexia with his illness but, unfortunately, no liver biopsy study for the presence of fat was done on this patient.

The authors conclude that infectious hepatitis does not show evidence of lipotropic

factor deficiency as revealed by this technic. This confirms the previous pathologic studies which have demonstrated that fatty infiltration is not a significant finding in the liver of infectious hepatitis patients. The contrast between cirrhosis and hepatitis in regard to phosphatide metabolism and possible deficiency of choline or its precursors appears to be verified.

Liver dysfunction is also often found in patients with cardiac failure and in the similar syndromes of adhesive and constrictive pericarditis. The nature of this dysfunction has been studied in relation to lipotropic factor deficiency in one patient by C. McMurray, D. Cayer, and W. E. Cornatzer (*Gastroenterology* **17,** *294 (1951)*). Their subject was a 38-year-old male who had had migratory arthritis four years prior to entry and eighteen months' history of cardiac failure symptoms. For twelve months he had had liver enlargement, ascites, skin pigmentation, and weight loss. No abnormality of carbohydrate metabolism was found. Biopsy of the liver, however, was reported to be compatible with diabetes mellitus due to the presence of glycogen deposits, but was not definitely diagnostic. Hemochromatosis was not present in either liver or skin biopsies. Liver function tests were markedly abnormal.

Phospholipid turnover studies following a large single dose of choline were carried out two weeks before and four weeks after pericardiolysis with the removal of a thickened adherent pericardium. There was marked improvement after surgery, with reduction in liver size and loss of ascites and edema. The turnover studies revealed an increase in specific activity of the plasma phospholipids which was greater before surgery than after. This finding the authors attribute to a possible choline deficiency which was corrected by lipotropic agents given throughout the hospital course. Liver biopsy taken at the time of the second phospholipid study revealed persistent cellular damage but fewer intracellular glycogen deposits.

Although the latter of these two reports suggests that choline deficiency can occur in the absence of marked fatty infiltration of the liver, it is difficult to evaluate the effect which the surgery and subsequent improvement in hepatic circulation had upon the metabolism of both phospholipids and lipotropic agents. Certainly no valid conclusion can be drawn since the two significant therapeutic measures, lipotropic factors and surgery, were introduced between the two studies. Another measurement of phospholipid turnover, taken after lipotropic agent therapy and before surgery might have been more enlightening. In view of these results, however, it would be of interest to carry out such studies in patients with cardiac failure where a similar hemodynamic situation involving hepatic enlargement exists.

The technic of measuring phosphatide metabolism used in these investigations is of definite value, but is handicapped by limitations in the frequency with which radioactive phosphorus studies can be carried out. It is impossible from the data available to determine what dose of choline or precursors will correct the apparent deficiency in some patients with cirrhosis. Similarly, it is not yet clear whether choline- or methionine-containing foods could substitute for the pure substances in producing this effect. The previous studies (*Nutrition Reviews, loc. cit.*) have shown that the increase in phospholipid turnover following lipotropic agents is not demonstrable after several months of dietary and lipotropic agent therapy. It is thus apparent that conclusions as to the necessity for pure choline or methionine in addition to a nutritious diet in the treatment of cirrhosis or pericarditis must be held in abeyance. In the majority of patients with hepatitis, however, lipotropic agents appear to be without benefit.

NUTRITIONAL STATUS AND BODY COMPOSITION

In recent years increasing attention has been paid to the quantitative evaluation of body composition, with special reference to fatness (*Nutrition Reviews* **9,** *344* (*1951*)). Work has been proceeding in several centers of research which at times lacked contact with each other. The lack of acquaintance with American work in this area is patent in a British study reported by R. A. McCance and E. M. Widdowson (*Proc. Roy. Soc.* (*London*) *B* **138,** *115* (*1951*); *Nutrition Reviews* **9,** *299* (*1951*)) and concerned with the breakdown of body weight of living man into its principal components, including body fat.

In the United States estimation of total body fat, in the context of nutritional research, has been based principally on specific gravity of the body. Application of this technic to problems of prolonged undernutrition was reported by J. Brozek (*Fed. Proc.* **5,** *13* (*1946*)). Changes in plasma volume and extracellular (thiocyanate) space during semistarvation and nutritional rehabilitation were studied by A. Henschel, O. Mickelsen, H. L. Taylor, and A. Keys (*Am. J. Physiol.* **150,** *170* (*1947*)). A comprehensive discussion of the changes in the main compartments of the body is to be found in the monograph by A. Keys *et al.* ("The Biology of Human Starvation," *Univ. of Minnesota Press, Minneapolis* (*1950*)), published after McCance and Widdowson's paper had been submitted for publication.

A formula for estimating the fat content from total body water, in addition to specific gravity, was presented by N. Pace and E. N. Rathbun (*J. Biol. Chem.* **158,** *685* (*1945*)); the percentage of fat = 100 − (percentage of water/0.732). Several methods for the measurement of body water *in vivo* were developed using deuterium oxide (heavy water), tritium (radioactive hydrogen), and particularly antipyrine. The literature was reviewed by J. Brozek and A. Keys (*Nutrition Abstr. Rev.* **20,** *247*, *see esp. p. 253* (*1950/51*)). The total body water (deuterium oxide method) and the extracellular fluid volume in 2 normal males and in 4 patients with protein depletion were determined by V. Hollander, P. Chang, and Co Tui (*J. Lab. Clin. Med.* **34,** *680* (*1949*)).

McCance and Widdowson (*loc. cit.*) determined total body water by measuring the concentration of urea in the blood serum. After samples for the measurement of the basal level of urea in body fluids and the endogenous excretion of urea in the urine were obtained, the subject took by mouth 25 g. of urea dissolved in water. Eighty minutes later a blood sample was drawn and urine collected. It was assumed that at this time the urea reached an equilibrium throughout the body fluids. Making proper corrections, the amount of ingested urea remaining in the body and the volume of water in which this urea was distributed were calculated. The extracellular fluid volume was determined by measuring the concentration of sodium thiocyanate in the blood serum two hours after injection of a known amount of this substance.

The difference between the volume of total body water and of extracellular fluid yielded the volume of water in the cells. From this value the weight of cellular tissue was calculated, assuming that water constituted 67 per cent of the weight of the cells. The total minerals in the body of normal persons represent a relatively constant fraction of the fat-free body mass. The mineral content was estimated to be equivalent to 7.5 per cent of the weight of the extracellular fluid plus the weight of the cell mass. The body fat was calculated by difference; fat = body weight − (extracellular fluid + cell mass + minerals).

This method of analysis was applied to small samples of "normal" and obese men and women as well as to 10 ex-prisoners-of-war who returned moderately undernourished from a Russian camp and were studied

in the course of nutritional rehabilitation. The average age of the first 4 samples varied from 30 to 41 years of age. The 7 normal men were slightly underweight in terms of height-weight standards, with an average relative weight (actual as percentage of standard) of 95; their bodies were estimated to contain 10.5 kg. (15.8 per cent) of fat, with a range from 7.2 to 22.4 per cent. In 3 grossly obese men, with the average relative weight of 181 per cent, the fat amounted to 46.3 kg. (37.9 per cent). In the two samples of "normal" and obese women the values were 111, 13.4 kg. (20.2 per cent) and 191, 54.0 kg. (48.8 per cent), respectively. These average values were calculated by the reviewer from the tabular data. The tables also give the values for extracellular fluid and cell mass. In the group of "normal" men, the extracellular fluid accounted for 25 per cent of the lean (fat-free) body mass. It represented 21 per cent of the total body weight.

The ten repatriated German soldiers before rehabilitation were 15 per cent below the weight standard for their height. The body fat decreased to 5.1 kg. (8.9 per cent) while the extracellular fluid increased to 22.2 kg. (38.6 per cent of body weight). The relative increase in the extracellular hydration, even in the absence of clinical edema, was noted also in the Minnesota semistarvation experiment with a rise in thiocyanate space from 23.5 to 34.0 per cent of body weight after six months of drastically reduced food intake (Henschel *et al.*, *loc. cit.*).

During rehabilitation, McCance and Widdowson's subjects increased in weight from 57.5 to 73.0 kg. in two months, becoming somewhat "overweight" (108 per cent of the standard). At the same time they gained a great deal of fat; the fat compartment was estimated at 15.3 kg. (20.2 per cent of body weight). A decrement in the extracellular fluid was reported, both in absolute (from 22.2 l. to 17.4 l.) and relative terms (38.6 to 23.9 per cent). The cell mass increased from 26.3 to 36.4 kg. This represents an increment of 38 per cent while the increment in fat corresponds to 200 per cent of the initial value. The "fattening" during refeeding after a period of food deprivation has been observed repeatedly and was documented in detail by Keys *et al.* (*loc. cit.*). The values for "active tissues" (cells) of the Minnesota subjects were 39.9 kg. during the control period, 29.2 after six months of starvation, and 39.8 kg. fifty-eight weeks after the end of semistarvation. These values represent the differences between total body weight and the sum of thiocyanate space, fat, and bone mineral (taken as 4 per cent of the pre-starvation body weight). In McCance and Widdowson's procedure it was the fat content which was obtained by the difference method.

They offer also a simpler method according to which the fat = body weight − (body water × $\frac{100}{71}$). The equation is identical with that proposed by Pace and Rathbun (*loc. cit.*), except that these authors calculated fat as percentage of body weight rather than as weight (kilograms). The difference in the numerator is due to a different estimate of the water content of the lean body mass—71 per cent and 73.2 per cent, respectively. Empirical data obtained for normal and obese subjects fit closely McCance and Widdowson's equation relating the water and fat content of the body. However, these investigators point out that the simplified method, not making individual corrections for mineral content, will give a valid estimate of fat content only for adults with a normal composition of the fat-free body mass. For young children who contain more extracellular fluid per kilogram of body weight than adults and for adults with disturbed hydration the method involving the determination of the extracellular fluids and cell mass is recommended.

For purposes of nutritional research on man, methods providing for the study of separate components of the body weight in living persons represent highly important tools of research. Attempts to validate some of the indirect procedures and to derive

"constants," such as the water content of lean body mass, needed in the estimation equations lead logically to the direct (chemical) study of the composition of the human body. In spite of the fundamental importance of such data, the available information is very meager and some of it goes back to J. Moleschott ("Physiologie der Nahrungsmittell: Ein Handbuch der Diätetik," *Giessen (1859)*). A detailed study of the chemical composition of an adult man has been made by H. H. Mitchell, T. S. Hamilton, F. R. Steggerda, and H. W. Bean (*J. Biol. Chem.* **158,** *625 (1945)*). The extractable fat accounted for 12.5 per cent of the body weight.

Recently an analysis of the bodies of 2 men, one middle-aged woman and one male child was reported by E. M. Widdowson, R. A. McCance, and C. M. Spray (*Clin. Sci.* **10,** *113 (1951)*). Granting the difficulty of obtaining bodies for analysis, it is unfortunate that 3 of the 4 patients were grossly abnormal, having died of uremia, infective endocarditis, and tuberculous meningitis, respectively. The female patient, who committed suicide by drowning, was the most "normal" of all. She was described as a masculine type but not wasted. On a fresh weight basis the percentages of nitrogen, protein, fat and water in the whole body were 2.3, 14.4, 23.6, and 56.0. Expressed as percentage of fat-free body mass, nitrogen, protein and water represented 3.1, 19.2, and 73.2 per cent.

The fat content of the 4 cadavers varied from 1.1 to 23.6 per cent. The total body water, on a fat-free basis, corresponded to 69.5 per cent in the child and 72.5 in the uremia patient. It rose to 82.0 in the patient with endocarditis; his body was wasted and edematous. The fat content in this man was very low (1.1 per cent). The authors comment that this case may serve to illustrate the hypothesis that, as the fat disappears in undernutrition, the spaces occupied by it in many parts of the body may be filled by (extracellular) fluid. It has been known for over one hundred years that as fat is removed from fat depots during starvation, it is replaced, in part at least, by water.

O. Bozenraad (*Deutsch. Arch. klin. Med.* **103,** *120 (1911)*) obtained a water content of 12.2 per cent for abdominal adipose tissue of well-nourished individuals and 31.0 per cent for emaciated persons. He also referred to Chossat's observations of 1838 indicating that the adipose tissue, together with other tissues, shows an increased water content in starvation. The inverse relationship between the fat and the moisture content of adipose tissue is clearly indicated by O. Schirmer's data (*Arch. f. exp. Pathol. u. Pharmakol.* **89,** *263 (1921)*).

In addition to nitrogen, protein, fat and water, Widdowson *et al.* (*loc. cit.*) analyzed the bodies for a variety of mineral constituents (calcium, phosphorus, sodium, potassium, magnesium, iron, copper, zinc) and for some amino acids (arginine, lysine, histidine, tyrosine, glutamic acid and ornithine). The values obtained by amino acid analyses for the 3 adults were very similar to each other. Furthermore, a comparison with data for 2 full-term infants seems to indicate that the amino acid make-up of the body does not change significantly with age.

Valuable comparative data on the chemical composition of adult man and adult animals of several species were provided by C. M. Spray and E. M. Widdowson (*Brit. J. Nutrition* **4,** *332 (1950)*). The weight, in kilograms, varied from 0.35 for the rat, 2.6 for the rabbit, 4.0 for the cat, to 125 for the pig; man's weight, considered "normal" for height, was given as 65 kg. The water content, as grams per 100 g. of fat-free tissue, varied within the narrow limits of 72.8 for man and rabbit to 75.6 for pig. The principal object of the study by Spray and Widdowson was the change in the chemical composition of mammals during growth and development from birth to maturity. In the species in which the sexes were studied separately, that is, in the rat and the rabbit, the adolescent and adult females were always fatter than

the males. In the pig, rabbit, and less markedly in the cat, there was a continued rise in the fat content of the body during the observed span of adult age. In the rat, observed up to ripe "old age," there was a steady increase in fat content, with a maximum reached at about 200 days of age (23.7 per cent in the female), followed by a decrease. The composition of these animals at birth has been reported by E. M. Widdowson (*Nature* **166,** *626 (1950)*). Some aspects of the body composition during pregnancy and lactation as phases of reproduction were studied in rats, mice and guinea pigs by C. M. Spray (*Brit. J. Nutrition* **4,** *354 (1950)*).

The systematic work involving a detailed chemical analysis of whole bodies and of some of the organs, carried out at the Medical Research Council Department of Experimental Medicine, University of Cambridge, has provided important additions to our knowledge of body composition. It is hoped that the work will continue unabated and that it will be feasible to add data on "normal" man. The Cambridge investigators are well aware of the fact that many more bodies need to be analyzed, especially for such highly variable constituents as body fat, before reliable figures for normal average and range of normal variation can be established. This information is needed as the basis for the study of abnormal developments in malnutrition, undernutrition and excess nutrition (obesity).

THE GLUCOSE EQUIVALENT OF FED PROTEIN

Conversion of protein to carbohydrate *in vivo* was pointed out in 1876 by the observations of S. Wolffberg (*Z. f. Biol.* **12,** *266 (1876)*) that glycogen was deposited in the livers of fowls first starved and then fed a carbohydrate-free meat powder. The quantitative interrelationships of this conversion have not, however, been unequivocally established in the seventy-five years since these observations. Studies in a variety of species of animals have been interpreted as indicating that roughly half of an average protein can be converted to carbohydrate. This conclusion was based on findings such as those of Minkowski and Lusk, and others since, who observed that when diabetic animals are fed only protein, or starved, there is a fairly constant ratio between urinary glucose and nitrogen. It was assumed that glucose formed under these conditions could come only from protein, was not utilized, and therefore, the urinary glucose: nitrogen ratio thus obtained was a direct index of the degree of protein conversion to carbohydrate. The glucose:nitrogen ratios observed were about 2.8 for the pancreatectomized dog and 3.65 for the phlorizinized dog.

Extension of similar studies to the fate of ingested amino acids by Dakin and others led to the concept of sugar-forming amino acids (such as glycine and alanine) and of nonsugar-forming ketogenic amino acids (such as histidine and phenylalanine).

The viewpoint that the ratio of urinary glucose:nitrogen in diabetic animals ingesting only protein is a direct measurement of the conversion of protein to carbohydrate has recently been criticized by R. W. Bancroft and D. R. Drury (*Am. J. Physiol.* **166,** *213 (1951)*). They point out that it is known that animals made diabetic by phlorizin or by pancreatectomy utilize glucose to varying degrees (H. J. Deuel, H. E. C. Wilson, and A. T. Milhorat, *J. Biol. Chem.* **74,** *265 (1927)*; P. O. Greeley and Drury, *Am. J. Physiol.* **130,** *249 (1940)*). Consequently, an estimation of the conversion of protein to carbohydrate must include a measure of glucose utilization, as well as of urinary glucose and nitrogen excretion.

Accordingly, Bancroft and Drury have

studied the problem in 2 phlorizinized depancreatized dogs. The dogs were first fed large amounts of (essentially) protein only (about 200 g. daily) and then fed only sucrose or glucose (100 g. daily). A processed dietary tunafish that contained about 28 per cent protein and less than 1 per cent carbohydrate and fat was supplemented with 50 to 100 g. of casein and used for the diet during the protein feeding period. Pancreatectomy was performed in addition to phlorization to overcome the possibility of a variable increased insulin secretion and consequent variable glucose utilization rate which might occur in the feeding of an animal with an intact pancreas. It was found necessary to administer a small (4 units) daily dose of insulin to prevent anorexia, vomiting and ketonuria. Urinary sugar and nitrogen were determined by standard technics. It was assumed that during the carbohydrate feeding period the differences between the quantity of sugar fed and that excreted indicated the amount of sugar utilized. The urinary glucose: nitrogen ratios observed on the protein diet were corrected for glucose utilized by an amount of sugar presumed utilized when glycosuria was roughly equivalent during the carbohydrate and protein feeding periods. By these methods Bancroft and Drury arrive at glucose:nitrogen ratios of 5.65 and 5.93, and glucose:protein ratios of 0.90 to 0.95. These figures are essentially the same as those calculated earlier by Drury in a study similarly conducted in dogs either phlorizinized or pancreatectomized (*J. Clin. Invest.* **21,** *153* (*1942*)).

In an earlier study D. R. Drury, H. C. Bergman, and P. O. Greeley (*Am. J. Physiol.* **117,** *323* (*1936*)) found that an average of 75 mg. of glucose per kilogram per hour were required to maintain the blood sugar at the preoperative level after hepatectomy in phlorizinized dogs. Analyses for lactic acid and muscle glycogen did not account for this glucose requirement. If the glucose utilized is assumed to come from protein and the observed urinary glucose: nitrogen ratio is corrected by this quantity, glucose:nitrogen ratios of the order of six are obtained. In keeping with these higher than classical figures (2.8 to 3.65) Bancroft and Drury cite the evidence of J. S. Butts, M. S. Dunn, and L. F. Hallman (*J. Biol. Chem.* **123,** *711* (*1938*)) that certain amino acids (histidine, isoleucine, tyrosine, and phenylalanine) formerly classified as not being convertible to glucose may lead to the deposition of glycogen. Further, they point out that D. R. Sanadi and D. M. Greenberg (*Arch. Biochem.* **25,** *257*, *323* (*1950*)) have shown that tyrosine, phenylalanine and tryptophan can be transformed in part to glucose.

Bancroft and Drury are correct in pointing out that the amount of glucose utilized by the test animals must be considered in addition to urinary excretions of glucose and nitrogen in estimating the conversion of protein to carbohydrate. However, it is not clear that this has been precisely estimated in their study. It should be noted that only 2 dogs were studied and that the test feeding periods were relatively short. It is not stated how soon after pancreatectomy observations were begun. Mean values only were used in their calculations, but deviations from the mean were marked in many instances. Further, the interpretation of their data involve the following assumptions: (1) that the degree of glycosuria during the period of protein feeding represented in the same proportions the available and utilized glucose as glycosuria of a similar degree in the carbohydrate feeding period; (2) that the same amounts of endogenous nitrogen were excreted during the various test periods; and (3) that the conversion of glycerol to glucose is the same during both the protein and carbohydrate feeding periods. None of these assumptions has been proved. In regard to the first, the time-course of glucose availability is different following a protein meal compared with

single-meal carbohydrate feeding—this may well influence the relative glucose utilization. The calculated values of the present study are, however, very similar to those of Drury's earlier findings mentioned above.

It would appear that significantly more protein is converted to carbohydrate than is indicated by uncorrected urinary glucose: nitrogen ratios of 2.8 to 3.65, but precisely how much more is not yet established. The importance of the adrenals in protein conversion to carbohydrate is well recognized. It is not known how closely results obtained in pancreatectomized-phlorizinized animals approximate the situation in normal animals. Investigation of the various metabolic pathways of carbohydrate and protein by means of labeled compounds and examination of various tissues and tissue components would facilitate further inquiry.

ACTION OF PYRITHIAMINE

One of the striking contributions to the physiologic chemistry of the vitamins has been the effect of antivitamins, compounds of similar chemical structure which antagonize the action of the vitamin concerned. One of these, pyrithiamine, is the pyridine analogue of thiamine; administered to a mouse consuming an experimental ration containing an adequate amount of thiamine, it produces all the symptoms of thiamine deficiency. Inasmuch as thiamine has been shown to be part of the cocarboxylase molecule and to exert its metabolic influence by way of this enzymatic action, it seems reasonable to expect that pyrithiamine may exert its antagonistic action at one or another point in the succession of changes of thiamine to the active enzyme, carboxylase.

Through the use of an enzymatic method, at least one important point of action of pyrithiamine has been shown (D. W. Woolley, *J. Biol. Chem.* **191,** *43 (1951)*). Although the tissue cells of most normal animals do not contain an enzyme which synthesizes cocarboxylase from thiamine, the blood of normal chickens does contain such a catalyst. By testing the washed red cells of the blood as well as the plasma, it was demonstrated that the cocarboxylase-synthesizing enzyme resides in the cells although it is active apart from the cell. The final analytic method employed provided not only for the synthesis of cocarboxylase from thiamine, but also for the combination of this coenzyme with apocarboxylase in washed yeast cells, the final measurement being made of the residual substrate (sodium pyruvate) after an incubation period of thirty minutes.

Using the foregoing method, it was observed that relatively large amounts of pyrithiamine decreased the formation of cocarboxylase by chicken blood enzyme from thiamine and could, if added in large enough quantity, completely stop the synthesis. Increasing the amount of thiamine in the system relieved the inhibition. Another point of possible interference by pyrithiamine is the combination of cocarboxylase with apocarboxylase (from the washed yeast cells) to form the complete enzyme system (carboxylase). When tested in the present study, the antimetabolite exerted no retarding influence upon the formation of carboxylase from cocarboxylase. However, it was observed that synthetic pyrithiamine pyrophosphate (corresponding to thiamine pyrophosphate or cocarboxylase) does antagonize both the combination of cocarboxylase with apocarboxylase and the reversal of this action. That this interference by pyrithiamine pyrophosphate plays little if any part in the reactions of the experimental model was shown by the demonstration that pyrithiamine pyrophosphate is not synthesized from pyrithiamine in chicken blood, but, indeed, is destroyed by chicken blood.

That the site of action of pyrithiamine may not be exclusively at the point of synthesis of cocarboxylase is shown by the fact that, while cocarboxylase is more effective in combating the toxic effects of the antimetabolites than is thiamine, as might be expected, pyrithiamine does show some toxic effect in mice even when cocarboxylase has been administered. Again, in the livers of mice to which pyrithiamine has been fed to the point of severe symptoms of thiamine deficiency, the concentration of cocarboxylase was not reduced from that in the livers of normal control animals, whereas it is known (S. Ochoa and R. A. Peters, *Biochem. J.* **32,** *1501 (1938)*) that in thiamine deficiency the cocarboxylase level in liver tissue is low. It is obvious that more work is needed to clarify the precise mechanism of action of the antivitamins.

GROWTH AND DEVELOPMENT OF NEGRO INFANTS

Since it has long been supposed that the lower birth weights found in the past in Negro infants in this country are in large part the result of inherited factors rather than a reflection of economic factors, the appearance of a study of the birth weight and growth rate during the first year of life of Negro infants living under relatively good economic conditions is of interest.

The birth weights of 11,818 Negro infants delivered in Freedmen's Hospital during the nine-year period 1939 to 1947 have been analyzed by R. B. Scott, M. E. Jenkins, and R. P. Crawford (*Pediatrics* **6,** *425 (1950)*). About one-half of these infants were classified as indigent. The average birth weight of the full-term male infants was 3378 g. with a standard deviation of 400 g. and that of the full-term female infants was 3269 g. with a standard deviation of 431 g. The incidence of prematurity was 9.5 per cent. These values compare quite favorably with the birth weights for white infants previously reported (E. C. Dunham, R. M. Jenss, and A. U. Christie, *J. Pediat.* **14,** *156 (1939)*; H. Bakwin, *Human. Biol.* **4,** *1 (1932)*) and are definitely larger than the birth weights for American Negroes included in the same two reports. There was little if any change in the average birth weight for Negro infants during the nine years of this study. The relatively higher birth weight observed in this series was attributed to more favorable economic conditions during this period of observation.

The relationships between birth weight, body length, epiphyseal maturation and economic status in 300 Negro infants were reported by A. Kessler and R. B. Scott (*Am. J. Dis. Child.* **80,** *370 (1950)*). The diameters of the distal femoral and proximal tibial epiphyses were measured. The previous findings of A. Christie (*Ibid.* **77,** *355 (1949)*) that these epiphyseal centers appeared earlier and were larger in girls than in boys of the same weight or length were substantiated. Two groups of mothers whose average daily milk intake during pregnancy had been one quart or more, or whose intake had been less than one pint, were studied separately, but no relationship between milk intake during pregnancy and the size of these centers was found. Nor was there a relationship to economic status as measured by the pay status at the hospital.

Growth curves for the first year of life on 654 Negro infants observed in private practice have been reported by R. B. Scott, W. W. Cardozo, A. deG. Smith, and M. R. DeLilly (*J. Pediat.* **37,** *885 (1950)*). Body weight was recorded at monthly intervals, and length every three months. Premature infants or those suffering from severe illnesses were excluded from this series. Charts of weight in pounds and height in inches against age for male and female Negro infants are included in this

report with the standard deviation of these observations outlined. When growth in height or in weight is plotted in this manner and compared with the data of R. M. Woodbury (*Children's Bureau Publication No. 87, Washington, D. C. (1921)*) the growth rates are definitely superior to the Woodbury standards for either white or Negro children and quite similar to those given by H. Bakwin and T. W. Patrick (*J. Pediat.* **24,** *405 (1944)*). The growth curve for Negro infants in this series is comparable to that for white children reported by H. C. Stuart (*J. Pediat.* **5,** *194 (1934)*).

It should be noted that the incidence of prematurity in this group of infants (9.5 per cent) is higher than that found in comparable groups of white infants. Since weight rather than maturation is commonly used as the criterion of prematurity, and since the studies leading to the somewhat arbitrary selection of a weight of 2500 g. as the dividing line were based primarily on white infants, it is quite possible that some smaller but physiologically mature infants were excluded from the present studies. If the greater maturity at any given birth weight of Negro infants as compared with white infants (A. Christie, *loc. cit.*) had been considered in defining prematurity in Negro infants it is quite possible that significant differences in birth weight based on race might be found.

These studies, as well as those of Bakwin and Patrick (*loc. cit.*), would suggest that under similar socioeconomic conditions the growth of Negro and white infants in the metropolitan areas of the United States is similar and that growth charts for use during the first year of life based on studies of white infants are probably applicable for use with Negro infants as well.

ROLE OF VITAMIN B_6 IN FAT METABOLISM

A number of experimental observations have indicated that vitamin B_6 may be concerned with the metabolism and storage of fat (H. Schneider, H. Steenbock, and B. R. Platz, *J. Biol. Chem.* **132,** *539 (1940)*; B. Sure and L. Easterling, *J. Nutrition* **39,** *393 (1949)*; *Nutrition Reviews* **8,** *36 (1950)*). However, this relationship has not been well defined nor the exact role of the vitamin determined. An attempt has been made to provide further information concerning the role of vitamin B_6 in fat metabolism in experiments recently reported by C. W. Carter and P. J. R. Phizackerley of the University of Oxford (*Biochem. J.* **49,** *227 (1951)*). In these experiments the total lipid of the body and the concentration of neutral fat, phospholipid and cholesterol have been determined in rats in which vitamin-B_6 deficiency had been induced. The results of these analyses are compared with those in pair-fed control animals. Evidence is presented indicating that in the rat vitamin-B_6 deficiency produces a decrease in total body lipid and in neutral fat of the liver.

Rats were fed one or the other of two highly purified basal diets deficient in vitamin B_6. The first was a low fat diet containing 4 per cent fat, while the second was a high fat diet which included 25 per cent fat. Calorie sources were provided by casein, sucrose, cod liver oil, and margarine. A balanced salt mixture was included in the ration. Each animal received a daily supplement of essential nutrients including thiamine, riboflavin, niacinamide, calcium pantothenate, para-aminobenzoic acid, biotin, inositol, choline, and arachis oil. Control animals received 40 micrograms of pyridoxine hydrochloride daily. The food consumption of each animal on the deficient diets was determined daily and an equivalent amount of food provided to the corre-

sponding paired control animal on the following day. The rats were killed when characteristic signs of dermatitis due to vitamin-B_6 deficiency had appeared. The time required for appearance of the typical skin lesions, and therefore sacrifice of the animals, varied from forty-six to one hundred and eighty-three days. The report does not so state, but it is concluded that the pair-fed controls were killed at the time of sacrifice of the corresponding animals on the deficient diet.

The effect of the various dietary regimens on the total body fat of rats in these experiments was of interest. The mean concentration of fat in deficient animals fed the low fat diet was 2.3 g. per 100 g. body weight as compared to 5.2 g. per 100 g. in rats provided the high fat diet, a difference shown to be of significance by statistical tests. The mean concentration of total body fat in the group of animals fed the low fat diet supplemented with 40 micrograms of pyridoxine daily was 8.5 g. per 100 g. body weight. In contrast, the pyridoxine supplement was without effect in increasing concentrations of body fat in rats which consumed the high fat diet. These data, therefore, demonstrate that vitamin-B_6 deficiency with diets of low fat content result in marked reduction in body fat.

Analysis of the data obtained from determination of lipid components in the livers of these animals demonstrated an effect of deficiency of vitamin B_6 in producing reduction of the neutral fat content in the liver. The concentration of neutral fat in deficient rats on the low fat diet was only one-half that of control animals, comparative values being 0.6 and 1.3 g. per 100 g. wet weight of liver. Similarly, the deficiency caused a reduction in the neutral fat content of the livers of animals on high fat intake. This is in contrast to lack of effect of vitamin-B_6 deficiency on total body fat when the diet contained larger proportions of preformed fat. There was no evidence that the vitamin or a deficiency of it influenced concentration of other lipids in the liver, *i.e.*, phospholipid and cholesterol.

The authors sought evidence of mechanisms which might be involved in reduction of total body fat and neutral fat of the liver in vitamin-B_6 deficiency. The first approach was that of investigation of the efficiency of intestinal absorption in deficient animals. No evidence was obtained of defects in absorption of dietary fat, protein or carbohydrate as determined by fecal fat excretion, nitrogen balance, and absorption of glucose from intestinal loops.

It was next considered that the effects of the deficiency might be accounted for if there were an acceleration of processes of enzymatic oxidation of lipid materials in the body. However, when the rate of oxidation of sodium butyrate and sodium octanoate by liver slices was determined, it was found that these processes were significantly depressed rather than accelerated.

The evidence in this report indicates a direct effect of vitamin B_6 on the deposition of fat in the body and of neutral fat in the liver. It confirms the earlier report of Sure and Easterling (*loc. cit.*) concerning loss of body fat, as well as the observations of H. W. Deane and J. H. Shaw (*J. Nutrition* **34,** *1 (1947)*) which revealed the absence of histologically demonstrable lipid in the liver in vitamin-B_6 deficiency. An explanation of this action of the vitamin has not been achieved. Evidence is presented which indicates that the effect is not one of impaired digestion and absorption nor of increased rate of oxidation of lipids. The explanation is offered that there is failure of fat synthesis in vitamin-B_6 deficiency. Certain of the data presented would support this conclusion. However, definitive proof of a role for the vitamin in fat synthesis is not offered. The present investigations might profitably be extended into areas of function of those enzymes which are integrally related to the synthesis of fat in the animal body.

CAROTENE SYNTHESIS IN TOMATOES

Beta-carotene is the principal vitamin A-active carotenoid found in tomato fruit, and indeed in most plant parts used for animal or human food. Although analytic methods for its measurement are now reliable, rapid, and in general use, the chemical steps involved in the formation of this important pigment have remained obscure. Studies extending over almost a decade, designed to improve the vitamin A potency of tomato fruits, have disclosed a wealth of information on the occurrence and structures of numerous simpler carotenoid compounds related to beta-carotene. From these experiments a reasonably detailed mechanism of carotene biosynthesis has been postulated by J. W. Porter and R. E. Lincoln (*Arch. Biochem.* **27,** *390 (1950)*).

	PARTS PER MILLION	VITAMIN A ACTIVITY
lycopene	300–400	no
beta-carotene	120	yes
tetrahydrolycopene	—	*
gamma-carotene	11	yes
delta-carotene	40	no
prolycopene	26	no
protetrahydrolycopene	16	no
zeta-carotene	85	no
alpha-carotene	9	yes
phytofluene	30	no
phytoene	43	*
tetrahydrophytoene	25–50	*

* Not bioassayed but probably not active.

One of their objectives was the development of tomato selections having a high content of each of the carotenes and colorless polyenes found in selections of *Lycopersicon*. Selections assumed to be homozygous for certain carotenes were found only for lycopene and beta-carotene, in which cases these pigments accounted for 95 to 100 per cent of the carotenes present. The above table presents the maximum contents of carotenes and polyenes found in selections bred for a high content of these compounds. Their vitamin A activities are of nutritional interest.

Only the selections high in beta-carotene are of practical nutritional significance.

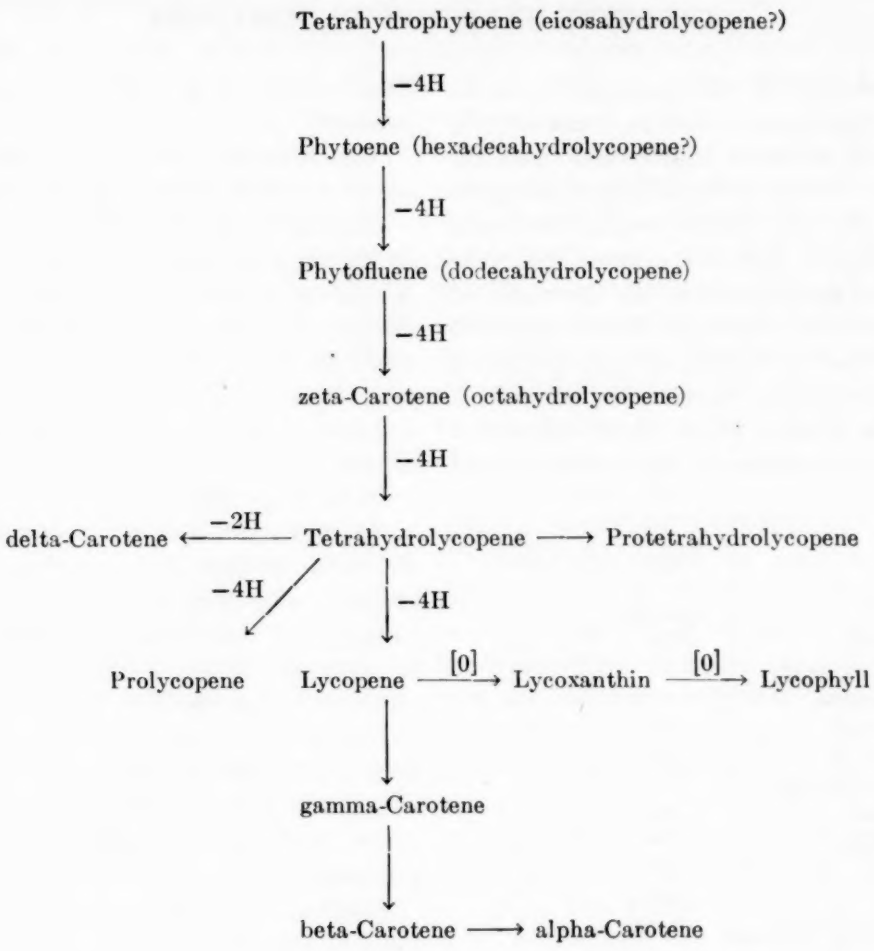
Commercial tomato varieties average 90 to 120 parts per million (p.p.m.) of lycopene. A mutant of Rutgers contained 150 p.p.m. Except for lycopene and beta-carotene, other carotenes listed occur in the presence of a complex mixture of many carotenes and constitute only 10 to 70 per cent of the mixture.

Three major genes, R, T, and B, are known to be involved in tomato carotene synthesis. For beta-carotene all three are necessary, for lycopene only R and T, and for synthesis of pigments of the Tangarine tomato, only R is required. If R is absent, a very low carotene content results. The Tangarine and Golden Jubilee varieties represent a homozygous type containing no lycopene but abundant prolycopene, protetrahydrolycopene, tetrahydrolycopene, zeta-carotene, phytofluene and phytoene.

Dominance of the factors for lycopene formation implies that lycopene is formed by dehydrogenation of other compounds as presented in the scheme on page 24.

The order of the compounds shown is on the basis of chemical structure. Evidence for the direction of the reactions is from studies on the inheritance of key compounds. Three types of reactions are involved: dehydrogenation, ring formation, and oxidation.

Since several of these compounds have been found in carrot roots and other yellow, orange, and red fruits and vegetables, it is probable that the same mechanism might apply for them. The scheme may not necessarily be valid for green leaves, though more exhaustive work might disclose small quantities in leaves. Tetrahydrophytoene has been found in leaves. This work suggests new experiments designed to study the



individual genes and enzymes involved in particular steps of the synthesis.

Inheritance studies show that a single gene difference exists between selections homozygous for lycopene and those for beta-carotene, as reported by R. E. Lincoln and J. W. Porter (*Genetics* **35,** *206 (1950)*). These workers studied crosses between these types. The high beta-carotene parent had a fruit size of 35 g. and contained 115 p.p.m. of beta-carotene, which was 96 per cent of the total carotenes. The low beta-carotene parent had fruit 180 g. in weight and contained 112 p.p.m. of total carotenes (mostly lycopene), of which only 6 per cent was beta-carotene. Study of the F_1, F_2, and F_3 populations indicated a difference between the two parents of a single incompletely dominant gene (B above), so far as carotene synthesis is concerned.

No evidence in the breeding program with either Rutgers or Indiana Baltimore as recurrent varieties indicated that increased beta-carotene content in the high selections is due to increase of total carotenes; instead, increased beta-carotene occurs at the expense of lycopene. A strong linkage exists between factors for high total carotene content and high lycopene. The beta-carotene content declined slowly as homozygosity of the selections was approached, indicating an association between a high beta-carotene percentage and a lower total carotene content.

It may be anticipated that extension of this study, both in tomato and other sources

of carotenes, will soon lead to great advances in our knowledge of how carotenoids are synthesized in nature. Such knowledge would help the plant breeder immeasurably by providing him with more specific tools for tracing the course of genes in his crossing experiments, leading more quickly to more nutritive varieties of food plants.

THE ENZYMATIC ACTION OF VITAMIN D

The biochemical mode of action of fat-soluble vitamins is still largely unknown. In particular, although many aspects of the over-all effects of vitamin D deficiency on calcium and phosphorus metabolism are known, no enzymatic interpretations of these effects could be given. A series of papers by R. Zetterström and his collaborators, working in Stockholm, Sweden, brings an important lead to the possible role of vitamin D in cellular physiology.

One obvious difficulty in studying the biochemical properties of any of the fat-soluble substances *in vitro* resides precisely in the fact that their lack of solubility in water prevents their interaction with purified water-soluble enzyme systems. Keeping in mind, however, the fact that other workers had been able to study the effects of alpha-tocopherol and of certain estrogenic hormones in the aqueous phase by preparing the phosphorylated derivatives, R. Zetterström (*Nature* **167,** *409 (1951)*) prepared phosphorylated vitamin D_2 (D_2P).

The vitamin is dissolved in dry, newly distilled pyridine, and phosphorylated by the action of phosphorus oxychloride in the dark at $-5°C$. The phosphorylated compound is soluble in water in concentrations up to 5×10^{-4} molar. It is characterized by its ultraviolet absorption spectrum, with a maximum absorption at 2620 to 2650 Å. It is considerably more sensitive to light than the nonphosphorylated substance, and upon irradiation certain physical properties, particularly its ultraviolet absorption, are reduced.

A family of enzymes of particular interest in rickets, and hence possibly concerned with the metabolic role of vitamin D, is that of the "alkaline phosphatases." These enzymes, in contradistinction to the "acid phosphatase" with an optimum pH of about 5, present their greatest rate of activity at a pH of about 9. They are widely distributed in ossifying cartilage, bone, kidneys, and intestinal mucosa. An alkaline phosphatase is also found, in relatively smaller amounts, in blood serum. It has been shown that phosphatases hydrolyze hexosemonophosphoric ester and glycerophosphoric ester, liberating inorganic phosphate. In the bone, it has been assumed that the inorganic phosphate thus liberated in turn affects the product of the calcium and phosphate ions in solution to such a degree that the solubility product is exceeded, and the excess calcium phosphate is deposited. Through a similar mechanism phosphatases might also play the essential role in intestinal absorption and kidney-tubule reabsorption of phosphorus. In rickets of infancy and early childhood the serum alkaline phosphatase is generally high. From a normal of 5 to 15 units per 100 cc. of blood, it may increase up to 20 to 30 units in mild cases, 40 to 60 units in moderately severe cases, and up to 190 units in very severe cases. Phosphatases appeared therefore to be the logical enzymes to investigate in connection with vitamin D metabolism.

With the new tool afforded by the water-soluble D_2P, R. Zetterström and M. Ljunggren (*Acta chem. Scandinav.* **5,** *283 (1951)*) were able to demonstrate that vitamin D activates alkaline phosphatases from the kidneys, intestines, and bones. When D_2P is added to purified kidney phosphatase, for example, in the presence of the classical substrate, phenylphosphate,

twice as much inorganic phosphate is split from the substrate during the first twenty minutes of incubation as appears without the addition of the phosphorylated vitamin. Similar results are obtained with intestinal phosphatase. In the case of bone phosphatase, the activating effect of D_2P is even higher, being of the order of 400 per cent during the first twenty minutes. The concentration of phosphorylated vitamin necessary for maximum activation is extremely small, being of the order of 10^{-5} molar. Maximum activation is reached at a high pH, approximately 9.7, close to the optimum pH of alkaline phosphatase.

The experimental results of Zetterström and Ljunggren seem thus to provide the key to an enzymatic explanation of the role of vitamin D in the prevention of rickets. If normal ossification is to take place, the animal must be able to absorb and retain a sufficient amount of phosphate and this must then be utilized by the bone-forming tissue. This in turn means that intestinal absorption, kidney tubule reabsorption, and phosphate deposition in the bone must be adequate. It has been recalled above that all three operations most probably entail a role for the alkaline phosphatases. If these need to be activated by vitamin D, the role of this vitamin becomes clear. Conversely, a deficiency in vitamin D will produce, through the resultant lack of activation of alkaline phosphatases, poor intestinal absorption of phosphates, excessive renal loss of phosphates, and poor ossification, the three essential aspects of rickets.

The role of vitamin D seems, however, to be more general than the simple prevention of rickets. Besides the general effect of vitamin D in growth (G. Stearns, P. C. Jeans, and V. Vandecar, *J. Pediat.* **9,** *1 (1936)*), the vitamin has been repeatedly implicated in carbohydrate metabolism, in muscle tissue in particular. More specifically, numerous experiments seem to indicate that in vitamin-D deficiency there is an impairment of the oxidative phase of carbohydrate metabolism (L. Laszt, *Helvet. physiol. pharmacol. acta* **1,** *C44 (1943)*). To find an enzymatic interpretation of these observations, R. Zetterström (*Acta chem. Scandinav.* **5,** *343 (1951)*) has also examined the effect of his water-soluble form D_2P on the oxygen uptake of the respiratory enzyme complex in kidney mitochondria obtained from healthy rabbits and rats. This enzyme complex is known to catalyze all the reactions of the tricarboxylic acid cycle and fatty acid oxidation.

The mitochondria were prepared by high speed, low temperature centrifugation of ground tissue under the proper conditions. They were then suspended in a medium containing potassium, magnesium, fluoride and phosphate ions; adenosine-5-phosphate was added; glutamate was used as oxidizable substrate, and glucose served as phosphate acceptor. Hexokinase was added in order to transfer phosphate to glucose. The pH of the mixture was 7.3, the temperature 30°C. The effect of D_2P, at a concentration of 2×10^{-5} molar on the oxygen uptake of the mitochondria suspension was then studied. Activation was found to be considerable during the initial period of the reaction. After thirty minutes of incubation, the oxygen uptake was found to be almost double that observed without the addition of D_2P. When the experiment was repeated using kidney mitochondria from rachitic, rather than healthy rats, the activation factor was much greater than 2.

As far as the mechanism of action of this activation of respiratory mechanisms is concerned, it did not at first appear that it is due to an increase in the rate of phosphorylation. In fact, net esterification of phosphate is shown to be less in the presence of D_2P than in its absence, and the difference gradually increases as the experiment is prolonged. Furthermore, the net uptake of phosphate decreases if the concentration of D_2P is increased. If on the other hand, phosphatases, as well as phosphorylases, are considered, this paradoxical effect becomes

understandable. The mitochondria contain a phosphatase which is specific for glucose-6-phosphate. After D_2P is added to the preparation, there is a marked initial increase in the phosphatase activity toward glucose-6-phosphate, similar to that observed toward phenylphosphate in the case of the purified alkaline phosphatase. The activating effect on respiration of the phosphorylated vitamin D, which takes place at the period of most intense esterification of organic compounds, is probably therefore obscured, when tested *in vitro*, by the concomitant activation of phosphatase activity, which masks the phosphate uptake. To the interpretation given in this earlier paper of the role of vitamin D in the prevention of rickets the author thus adds a clear basis for the understanding of the role of vitamin D in general metabolism.

When the year's progress in nutrition is reviewed, it seems possible that the work of Zetterström, if confirmed, will be considered an outstanding contribution. Not only does the enzymatic role of vitamin D become understandable, but furthermore, this enzymatic role permits one to interpret all of the essential symptoms of vitamin D deficiency. Thus, an explanation of the intestinal and renal aspects of vitamin D deficiency, an interpretation of the essential aspects of rickets as far as bone formation is concerned, and a suggestion of the possible role of vitamin D in general metabolism all become available. By comparison, it may be recalled that even in the cases of B vitamins for which one or several enzymatic functions have been known for several years, this knowledge does not yet afford a rational explanation of the observed effects of the corresponding deficiency.

IN VIVO SYNTHESIS OF METHYL GROUPS

The term "labile methyl group" has become a familiar byword in the language of intermediary metabolism and is taken to mean the methyl group which appears to be readily transferred from a methyl donor to a methyl acceptor in certain important biochemical reactions. The significance of this transmethylation reaction is indicated by several examples of its occurrence. Thus, in the well-known lipotropism brought about by methionine, it appears that this amino acid provides the methyl groups for the formation of choline, under the influence of which excess hepatic fat is removed, probably as phospholipid. Again, several types of evidence show that the methyl group in endogenous creatine is transferred to glycocyamine from methionine. Furthermore, cessation of growth of young animals brought about by removal of methionine from the diet can be prevented if choline (as a methyl donor) and homocystine are present. These observations were looked upon as a confirmation of the early suggestion by Hofmeister that the animal must secure preformed methyl groups in the diet in order to enjoy optimal nutrition.

However, some time later it was reported (V. du Vigneaud, S. Simmonds, J. P. Chandler, and M. Cohn, *J. Biol. Chem.* **159,** *755 (1945)*) that when the content of deuterium in the body water of rats maintained on an adequate diet was raised to approximately 3 per cent, the methyl groups in the choline from the body tissues contained enhanced amounts of deuterium, indicating some synthesis of methyl groups. A still more striking evidence of *in vivo* synthesis of methyl groups was indicated (M. A. Bennett, G. Medes, and G. Toennies, *Growth* **8,** *59 (1944)*) by the growth of some of the rats restricted to a purified ration containing homocystine but devoid of a source of labile methyl groups. One obvious source of the implied endogenous methyl groups resided in the intestinal flora of the

experimental animals, but this was ruled out by employing germ-free rats in which there still was evidence of the synthesis of methyl groups (du Vigneaud, C. Ressler, and J. R. Rachele, *Science* **112,** *267 (1950)*). The foregoing *in vivo* evidence has been supported by *in vitro* experiments (W. Sakami and A. D. Welch, *J. Biol. Chem.* **187,** *379 (1950)*) in which C^{14}-labelled formate was added to the medium in which rat liver slices were maintained and the radioactivity demonstrated in the methionine and choline isolated from the tissue.

A logical extension of the previous studies has been the search for compounds from which biologically labile methyl groups are synthesized in the animal body. In a series of experiments recently reported (V. du Vigneaud *et al.*, *J. Am. Chem. Soc.* **73,** *2782 (1951)*), methyl alcohol, sodium bicarbonate, sodium formate and formaldehyde, all labelled with C^{14}, were injected into rats and the choline prepared from the animal tissues was examined for C^{14}. In each case, save that of bicarbonate, it was demonstrated that the rat can utilize the appropriate compound for the biosynthesis of labile methyl groups. In the case of methyl alcohol, one of several interpretations may be made: the intact methyl groups might be utilized in the synthesis of one of the compounds in the transmethylation series; the methanol might be an actual metabolic intermediate in the transfer of methyl groups; or the alcohol, through oxidation and then reduction, might act as a precursor of the methyl group of choline, indirectly. By labelling the methyl group of the methanol with both deuterium and C^{14}, and comparing the ratios of C^{14} to deuterium in the labelled methanol injected with that in the methyl group of the derived choline, it was demonstrated that the methyl group of the alcohol is not used intact, but serves as a precursor of the biologically labile methyl group.

Further studies in which C^{14}-formyl-L-phenylalanine was injected or C^{14}-methyl stearate was fed to rats indicate that the carbon of the formyl radical, and the carbon of the methyl group of the ester, appear in the methyl group of the tissue choline. It has thus been shown that, contrary to the early concept that methyl groups must be provided as such for the needs of the animal, this group can be of endogenous origin, arising from other one-carbon compounds.

FLUORIDE CONTENT OF TEETH

Studies continue to be reported which gradually increase our knowledge of the effect of fluoride consumption on the development of teeth which are highly resistant to tooth decay. Two recent surveys have examined the effect of fluoride consumption in nations which normally have a much lower caries incidence than is to be found in the United States, Great Britain, and the Scandinavian countries. In the first of these studies, T. Mavrogordato (*J. Dent. Res.* **30,** *225 (1951)*) has described the results obtained in dental surveys conducted in Greece in which the incidence of tooth decay and mottled enamel were determined for individuals in Lavrion where the water contained 3.2 parts per million (p.p.m.) of fluorides and for individuals in other parts of Greece where the water contained low or negligible amounts of fluoride. Some 4000 children were examined in the 6 low-fluoride districts. The percentage of 5- to 18-year-old children with tooth decay varied from 33 to 70 per cent in the various communities. Large variations in frequency of tooth decay existed between these communities but, as will be seen below, there was no overlapping with the

data from Lavrion. Furthermore, the frequency of tooth decay was extremely low as compared with the incidence in low-fluoride areas in the United States. However, in Lavrion only 4.81 per cent of 291 children 6 to 12 years old, who were born there or moved there before 1 year of age, had any carious teeth. There was an average of only 44 decayed teeth per 100 children. One hundred and ninety children, 12 to 18 years old, who grew up in Lavrion had 135 carious teeth per 100 children with an average of 77.7 per cent of the individuals being caries-free. The deciduous teeth of the children born or raised in Lavrion were free from mottled enamel; this observation confirmed previous statements to the effect that insufficient fluoride passed across the placenta to cause mottling of the deciduous teeth. In contrast, the enamel of the permanent teeth of the children raised in Lavrion were mottled to varying degrees. Children of comparable ages who moved into Lavrion after tooth development had been largely completed had appreciably more tooth decay than the groups born and raised in Lavrion; however, their caries incidence was much lower than that of children raised in other regions of Greece where the fluoride levels were negligible.

In the second report, as the result of a similar survey in Hungary where the caries incidence also is remarkably low in low-fluoride areas as compared with similar low-fluoride areas in the United States, P. Adler (*J. Dent. Res.* **30,** *368* (*1951*)) reported the data obtained in 8 communities. One thousand and four 12- to 14-year-old children were examined in 4 communities where the fluoride content of the waters varied from 0.72 to 1.09 p.p.m. The average number of decayed, missing and filled (DMF) permanent teeth per 100 children was 111. Approximately 50 per cent of the children had no diseased teeth. Seven hundred and eight children of the same age were examined in 4 areas where the fluoride content of the water varied from 0.20 to 0.33 p.p.m. The average number of DMF permanent teeth was 284 per 100 children and about 21 per cent of the children were caries-free. The caries incidence in the 4 low-fluoride communities was remarkably lower than among children in the low-fluoride areas of this country. However, even though the caries incidence was very low in low-fluoride areas, the increased ingestion of fluorides in similar communities where fluorides occurred in the drinking water at levels around 1 p.p.m. had resulted in appreciable reduction in the caries incidence.

These studies by Mavrogordato and Adler are of particular interest in the nutritional field because they clearly indicate that there are other factors than fluorides which must be considered and evaluated.

In order to be able to understand more about the role of fluorides in the dental structures, we need to have a greater knowledge of the amount of fluorides in the teeth, the form in which they are present and also the routes by which they entered and were incorporated there. In one exploration of this type, F. J. McClure and R. C. Likins (*J. Dent. Res.* **30,** *172* (*1951*)) have reported an extensive series of fluoride analyses on the enamel and dentin of teeth from individuals with continuous residence in communities which had a wide range of fluoride concentrations in the drinking water supplies. No difference was observed between the fluoride content of the enamel or of the dentin from carious and noncarious teeth. This observation is contrary to previous studies in which the fluoride content of enamel and dentin from carious teeth was reported to be lower than the fluoride content of enamel and dentin, respectively, from noncarious teeth (*Nutrition Reviews* **5,** *322* (*1947*)). The fluoride analyses were conducted by acceptable scientific procedures. The average fluoride content of the enamel from 224 noncarious teeth from 33 individuals with continuous residence in the

neighborhood of Washington, D. C., where the drinking water supplies contained from 0.0 to 0.2 p.p.m., was 0.0102 per cent with a range from 0.0046 to 0.0203 per cent. The average for enamel from 202 carious teeth from the same individuals was 0.0098 per cent with a range from 0.0042 to 0.0220 per cent. The average fluoride concentration for the dentin from the 224 sound teeth was 0.0240 per cent, ranging from 0.0113 to 0.0482 per cent, and for the dentin from the 202 carious teeth was 0.0227 per cent with a range from 0.0086 per cent to 0.0517 per cent.

As a typical example of the increase in the fluoride content of enamel and dentin of teeth developed in Aurora, Illinois, where the drinking water contained 1.1 to 1.2 p.p.m., the average fluoride contents of enamel and dentin from 36 carious and intact teeth from 31 individuals 8 to 25 years of age were 0.0135 and 0.0360 per cent, respectively. In view of the fact that the water consumed during the development of these teeth contained 1.1 to 1.2 p.p.m. of fluoride, it is unlikely that these teeth could be considered to be significantly mottled (fluorosed). In contrast, the average fluoride content of 7 noncarious teeth from individuals in Chetopa, Kansas, where the water contained 7.6 p.p.m. of fluoride, was 0.0658 per cent for enamel with a range from 0.0384 to 0.1059 per cent, and 0.1277 per cent for dentin, varying from 0.0809 to 0.1814 per cent. Several other sets of data were presented for other communities with intermediate levels of fluorides in the communal water supplies. In all cases, there were rather high variations within the group which probably indicated that there were great differences in the amounts of fluoride ingested by individuals within any community, or that individuals varied considerably in their ability to metabolize and excrete the fluoride ingested.

In an experiment to test the increase in fluoride content in the enamel of teeth after topical fluoride application, A. B. Hord and R. G. Ellis (*J. Dent. Res.* **30,** *360 (1951)*) applied a 2 per cent sodium fluoride solution to previously cleaned vital teeth in three young dogs. The applications were made to the posterior teeth in the upper right and lower right quadrants; the left quadrants were maintained untreated as controls. Each exposure to the fluoride solution lasted ten minutes and was repeated at weekly intervals for eight weeks. The dogs were killed after the last treatment and the teeth extracted and fixed in 10 per cent formalin until the fluoride analyses were made. No absolute separation of the enamel and dentin was attempted because of the thinness of the enamel in dog teeth. The samples analyzed consisted of the enamel and just enough dentin to hold the enamel rods together. The control samples contained an average of 0.034 per cent fluoride while the topically-treated samples contained an average of 0.095 per cent fluoride, *i.e.*, an increase of 2.79 times. The increases in fluoride content between bilaterally symmetrical teeth varied from 1.81 to 3.18 times the control values. The average level of fluoride in the samples from the control teeth was 0.034 per cent while the average amount in the treated teeth was 0.095 per cent. These investigators believed that the increase in fluoride content was due to the presence of an increased amount of calcium fluoride. They did not state how they differentiated between calcium fluoride and fluoroapatite in arriving at this conclusion. Insofar as is known, there are no other values for the fluoride content of the enamel of dog's teeth. The average level for the control samples reported by Hord and Ellis was much less than the lowest average values for human enamel given by McClure and Likins; however, the samples from fluoride-treated teeth had an average fluoride content comparable to the content of enamel from teeth of individuals who were raised in communities where there were not appreciable amounts of fluoride in the drinking water.

NOTES

Letter to the Editor

Dear Sir:

In a review in your June 1951 issue entitled "Urinary Excretion of Vitamin B_{12} in Human Beings" your reviewer says:

"One wonders what the results would be in the treatment of pernicious anemia if huge doses, up to several grams of vitamin B_{12}, were given orally without normal gastric juice."

This is exactly the work that C. C. Ungley has been carrying out in this country, except that doses of a few milligrams rather than a few grams of vitamin B_{12} (without gastric juice) sufficed to give excellent responses in pernicious anemia patients. This was reported in the *British Medical Journal* for October 21st, 1950, page 905.

You may be interested to know that my colleague Dr. W. F. J. Cuthbertson has also investigated the excretion of vitamin B_{12} by humans. His paper was presented to the Biochemical Society in January of 1951 and appears in abstract form in the April 1951 number of the *Biochemical Journal* (D. C. Chesterman, Cuthbertson, and H. F. Pegler, *Biochem J.* **48,** *proc. li*). Unlike Dr. Ungley's paper, this would not have been available in America when your review was written.

E. Lester Smith
Glaxo Laboratories Ltd.
Greenford, Middlesex
England

Copper Metabolism

A symposium on copper metabolism was held at the Johns Hopkins University under the sponsorship of the McCollum-Pratt Institute in June, 1950. The papers and discussion presented at this meeting are published in "Copper Metabolism. A Symposium on Animal, Plant and Soil Relationships," edited by W. D. McElroy and H. B. Glass, *The Johns Hopkins Press, Baltimore (1950)*.

The following aspects of the subject were presented: the formation of copper complexes; the copper protein, ascorbic acid oxidase; the nature of the copper enzymes involved in tyrosine oxidation; phenol oxidase and plant respiration; functional aspects of copper in plants; the copper nutrition of green plants and fungi; a browning reaction involving copper proteins; copper metabolism in the invertebrates; hemocyanin; the use of radioisotopes of copper and molybdenum in nutritional studies; the influence of copper on the metabolism of phosphorus and molybdenum; problems associated with copper deficiency in ruminants; copper and molybdenum in relation to diseases of cattle and sheep in New Zealand; copper metabolism in human subjects; the chemical nature of the copper complexes in peat soils and plants; trace elements and phosphate in herbage plant nutrition; the nutrient element content of native forages in relation to land forms and soil types in the North Carolina Coastal Plain.

Each of these areas of investigation was presented by a specialist who was chosen on the basis of his own research in the given area of study. The wide diversity of background of the speakers afforded an appreciable exchange of ideas which is recorded in the discussion following each chapter.

For those who are interested in the subject of copper metabolism this volume presents the most complete and up-to-date treatment of the topic which is now available in the literature. The final chapter consists of a summary of the symposium.

CLINICAL NUTRITION BRIEFS

"Deep burns (particularly of the 'wet' necrosis variety) may lead to marked nutritional disturbances. If the patient is maintained in good nutritional state, granulation tissue appears firm, red, and healthy, skin grafts take well, and donor sites heal uneventfully. Even if the area burned is very extensive, healing can take place if the patient is maintained in good condition and if infection is controlled." (*Nutrition Reviews* **9,** *257* (*1951*), Nutritional Problems of Patients with Burns.)

"This case is but a suggestive example of the possible nutritional effects of antibiotic administration to humans. With the increasing use of these agents over longer periods of time it may be expected that more such cases will be seen. Studies of vitamin metabolism and excretion in patients receiving these agents are clearly indicated." (*Nutrition Reviews* **9,** *283* (*1951*), Vitamin Deficiency Signs During Antibiotic Therapy.)

"An interesting analysis of the lipid content in normal and arteriosclerotic human aortas is reported. Thirty-one aortas were removed from males with ages distributed from birth to 85 years. The normal appearing aortic sections showed a linear increase with age in total cholesterol and its ester, and total phospholipid. The cholesterol esters increased somewhat faster with age than did the total cholesterol content." (*Nutrition Reviews* **9,** *295* (*1951*), Relation of Experimental Arteriosclerosis to the Disease in Man.)

"Of the 69 cases of scurvy detected at autopsy only 6 were recognized clinically. This finding is not too surprising when it is realized that some of these cases had not advanced to the point at which clinical recognition becomes possible. These studies suggest that scurvy is more prevalent in infants not receiving breast milk than had been suspected on the basis of the incidence of clinically recognized cases." (*Nutrition Reviews* **9,** *297* (*1951*), Prevalence of Scurvy During Infancy.)

"The data reported indicate deficient intakes of all the nutritional elements studied, although marked variations in intake of different elements in the same person occurred. It is to be emphasized that these elderly people not only showed signs of nutritional deficiency, but also suffered physical handicaps which may have served to emphasize their inability to eat, as well as increasing their requirements for the various nutrients." (*Nutrition Reviews* **9,** *304* (*1951*), Food Intake of Elderly Hospitalized Patients.)

"The relationship between food factors and blood coagulation in clinical conditions is acutely important in patients with gastrointestinal bleeding, usually originating from peptic ulcers. These workers have studied the effect of the oral administration of 20 per cent cream from cow's milk on the coagulation time." (*Nutrition Reviews* **9,** *325* (*1951*), Fat and Blood Coagulation.)

"These studies of absorption and intermediary metabolism using the portal anastomotic venous collaterals on the anterior abdominal wall suggest new methods for the study of intermediary metabolism in man, especially in diseases of the liver." (*Nutrition Reviews* **9,** *356* (*1951*), Portal Blood Investigations in Man.)